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TITLE OF THE INVENTION (500 characters max)

DIAGNOSIS AND MONITORING OF SYSTEMIC LUPUS ERYTHEMATOSUS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☐ Application Data Sheet. See 37 CFR 1.76

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Respectfully submitted,

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

DIAGNOSIS AND MONITORING OF SYSTEMIC LUPUS ERYTHEMATOSUS

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DIAGNOSIS AND MONITORING OF SYSTEMIC LUPUS ERYTHEMATOSUS

FIELD OF THE INVENTION

[01] This invention relates to the diagnosis and/or monitoring of patients with systemic lupus erythematosus, including methods and kits for carrying out this activity.

BACKGROUND OF THE INVENTION

[02] This invention relates to the diagnosis and/or monitoring of patients with systemic lupus erythematosus (SLE).

[03] Systemic lupus erythematosus (SLE) or lupus is the prototypic autoimmune disease resulting in multiorgan involvement. This anti-self response is characterized by autoantibodies directed against a variety of nuclear and cytoplasmic cellular components. These autoantibodies bind to their respective antigens, forming immune complexes which circulate and eventually deposit in tissues. This immune complex deposition causes chronic inflammation and tissue damage.

[04] Diagnosing and monitoring disease activity are both problematic in patients with SLE. Diagnosis is problematic because the spectrum of disease is broad and ranges from subtle or vague symptoms to life threatening multi-organ failure. There are other diseases with multi-system involvement that can be mistaken for systemic lupus, or vice versa. Criteria were developed for the purpose of disease classification in 1971 (Cohen, AS, *et al.*, 1971, Preliminary criteria for the classification of systemic lupus erythematosus. *Bull Rheum Dis* 21:643-648) and revised in 1982 (Tan, EM, *et al.*, 1982. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arth Rheum* 25:1271-1277.) and 1997 (Hochberg, MC. 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arth Rheum* 40:1725). These criteria are meant to ensure that patients from different geographic locations are comparable. Of the eleven criteria, the presence of four or more, either serially or simultaneously, is sufficient for classification of a patient as having SLE. Although the criteria serve as useful reminders of those features that distinguish lupus from other related autoimmune diseases, they are unavoidably fallible. Determining the presence or absence of the criteria often requires interpretation. If liberal standards are applied for determining the presence or absence of a sign or symptom, one could easily diagnose a patient as having lupus when in fact they do

not. Similarly, the range of clinical manifestations in SLE is much greater than that described by the eleven criteria and each manifestation can vary in the level of activity and severity from one patient to another. To further complicate a difficult diagnosis, symptoms of SLE continually evolve over the course of the disease. New symptoms in previously unaffected organs can develop over time. There is no definitive test for lupus and, thus, it is often misdiagnosed.

[05] Monitoring disease activity is also problematic in caring for patients with lupus. Lupus progresses in a series of flares, or periods of acute illness, followed by remissions. The symptoms of a flare, which vary considerably between patients and even within the same patient, include malaise, fever, symmetric joint pain, and photosensitivity (development of rashes after brief sun exposure). Other symptoms of lupus include hair loss, ulcers of mucous membranes and inflammation of the lining of the heart and lungs which leads to chest pain. Red blood cells, platelets and white blood cells can be targeted in lupus, resulting in anemia and bleeding problems. More seriously, immune complex deposition and chronic inflammation in the blood vessels can lead to kidney involvement and occasionally failure requiring dialysis or kidney transplantation. Since the blood vessel is a major target of the autoimmune response in lupus, premature strokes and heart disease are not uncommon. Over time, however, these flares can lead to irreversible organ damage. In order to minimize such damage, earlier and more accurate detection of disease flares would not only expedite appropriate treatment, but would reduce the frequency of unnecessary interventions. From an investigative standpoint, the ability to uniformly describe the "extent of inflammation" or activity of disease in individual organ systems or as a general measure is an invaluable research tool. Furthermore, a measure of disease activity can be used as a response variable in a therapeutic trial.

[06] Two of the most commonly used instruments are the Systemic Lupus Disease Activity Index (SLEDAI) (Bombardier, C., D. D. Gladman, *et al.* (1992). Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arth Rheum* 35: 630-40), and the Systemic Lupus Activity Measure (SLAM) (Liang, M. H., S. A. Socher, et al. (1989). Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. *Arth Rheum* 32: 1107-18). The SLEDAI includes 24 items representing 9 organ systems. The variables are obtained by history, physical examination and laboratory assessment. Each item is weighted from 1 to 8 based on the significance of the organ involved. For example, mouth ulcers are scored as 2, while

seizures are scored as 8. The laboratory parameters that are included in the SLEDAI include white blood cell count, platelet count, urinalysis, serum C3, C4 and anti-dsDNA. The total maximum score is 105. The SLAM includes 32 items representing 11 organ systems. The items are scored not only as present/absent, but graded on a scale of 1 to 3 based on severity. The total possible score for the SLAM is 86. Both the SLEDAI and the SLAM have been shown to be valid, reliable, and sensitive to change over time (Liang, M. H., S. A. Socher, et al. (1989). Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. *Arth Rheum* 32:1107-18), and are widely used in research protocols and clinical trials. These indices are particularly useful for examining the value of newly proposed serologic or inflammatory markers of disease activity in SLE.

[07] Despite the obvious utility of these instruments, there are some drawbacks. First, there is not always complete agreement between the SLAM and the SLEDAI in the same set of patients. There are several possible reasons for these discrepancies. Unlike the SLEDAI, the SLAM includes constitutional symptoms such as fatigue and fever, which may or may not be considered attributable to active SLE; this activity index relies on physician interpretation. In addition, the SLEDAI does not capture mild degrees of activity in some organ systems and does not have descriptors for several types of activity such as hemolytic anemia. For these and other reasons, most studies incorporate more than one measure of disease activity.

[08] A general review of the state of the art can be found in Ramsey-Goldman, R. and Manzi, S. Systemic Lupus Erythematosus . In: Goldman and Hatch, Ed. Women and Health. Academic Press, San Diego, CA 2000: 704-723.

[09] The complement system consists of a complex network of more than 30 functionally linked proteins that interact in a highly regulated manner to provide many of the effector functions of humoral immunity and inflammation, thereby serving as the major defense mechanism against bacterial and fungal infections. This system of proteins acts against invasion by foreign organisms via three distinct pathways: the classical pathway (in the presence of antibody) or the alternative pathway (in the absence of antibody) and the lectin pathway. Once activated, the proteins within each pathway form a cascade involving sequential self-assembly into multimolecular complexes that perform various functions intended to eradicate the foreign antigens that initiated the response.

[10] The classical pathway is usually triggered by an antibody bound to a foreign particle. It consists of several components that are specific to the classical pathway and designated C1, C4, C2, (in that order in the pathway).

[11] In the classical pathway, the first component C1q is bound to an antigen-antibody complex, activating the pathway. This event is followed by sequential activation of the two serine proteases C1r and C1s. Activated C1s has two substrates, the final two proteins of the classical pathway, namely C4 and C2. Protein C4 is cleaved into C4a and C4b. Protein C2 is cleaved to form C2a and C2b. Fragments C4b and C2a assemble to form C4b2a, which cleaves protein C3 into C3a and C3b, which completes activation of the classical pathway.

[12] Fragments C4b and C3b are subject to further degradation by Factor I. This factor cleaves C4b to generate C4d and also cleaves C3b, to generate iC3b followed by C3d. Thus, activation of the classical pathway of complement can lead to deposition of a number of fragments, including C4d and iC3b on immune complexes or other activating surfaces. These fragments are ligands for complement receptor type 1 (CR1) on erythrocytes or red blood cells.

[13] There have been no reports regarding identification of complement protein C4 on surfaces of platelets obtained from the circulation of humans.

BRIEF SUMMARY OF THE INVENTION

[14] The invention involves the use of determinations of complement component C4d on surfaces of platelets obtained from the circulation of healthy individuals, individuals with systemic lupus erythematosus, and individuals with other diseases.

[15] In one aspect, this invention comprises a method of diagnosing systemic lupus erythematosus in an individual, comprising (a) determining, in a blood sample from the individual containing platelets, complement component C4d deposited on surfaces of platelets in the sample, and (b) comparing said determination with the quantity of component C4d deposited on the surface of platelets of individuals not having systemic lupus erythematosus.

[16] In a second aspect, this invention comprises a method of monitoring systemic lupus erythematosus in an individual, comprising (a) determining, in a blood sample from the individual containing platelets, complement component C4d deposited on surfaces of platelets in the sample, and (b) comparing said determination with the quantity of component C4d deposited on the surface of platelets previously obtained from the individual.

[17] The invention also comprises automated methods of the above types, computer software for performing such automated methods, and kits for performing the methods described herein.

DETAILED DESCRIPTION OF THE INVENTION

General discussion

[18] The methods of this invention enable the diagnosis and/or monitoring of SLE. Because this condition is a serious health problem, there is a need for relatively accurate and early diagnosis of this condition. Likewise, the ability to monitor the activity of this disease is of great importance.

[19] The invention involves the use of determination of complement component C4d on surfaces of platelets.

[20] In the most general sense, the methods of this invention are based on the discovery by the inventors that a determination of C4d deposited on surfaces of platelets of a patient can serve as a diagnostic marker for SLE.

[21] In diagnosing the occurrence, or previous occurrence, of SLE, complement component C4d deposited on surfaces of plalelets in a sample is determined. This determination is then compared with the quantities of C4d usually found on the surfaces of platelets of individuals not having SLE.

[22] In monitoring disease activity of a patient with SLE, the same determination is made in the patient's blood sample, and is then compared with determinations of the quantities of C4d on surfaces of platelets in a sample obtained from the same patient in the past.

[23] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

General procedures

[24] The invention involves conducting assays on blood samples obtained from patients to determine C4d.

[25] Samples of blood are obtained from the patient and are treated with EDTA (ethylenediaminetetraacetate) to inhibit complement activation. The samples are maintained at room temperature or under cold conditions. Assays are run preferably within 48 hours.

[26] The determination of C4d may be done by a number of methods including flow cytometry, ELISA using platelet lysates, and radioimmunoassay. In one embodiment of this invention, the determination of the level of C4d is made using flow cytometric methods, with measurements taken by direct or indirect immunofluorescence using polyclonal or monoclonal antibodies specific for C4d. The mean fluorescence channel (MFC) for platelet

C4d is determined. The same type of assay may be used for diagnosis or for monitoring disease activity in patients known to have SLE.

Kits

[27] Kits for conducting the assays for both the diagnosing of disease and monitoring of disease activity are a part of this invention. Said kits will use any of the various reagents needed to perform the methods described herein. For example using the immunofluorescence assays, the kits will generally comprise a conjugate of a monoclonal antibody specific for complement component C4d with a fluorescent moiety, and preferably also a conjugate of a monoclonal antibody specific for platelet CD42b with a different fluorescent moiety. Additionally, the kits will comprise such other material as may be needed in carrying out assays of this type, for example, buffers, radiolabelled antibodies, colorimeter reagents etc.

[28] The antibodies for use in these methods and kits are known. Hybridomas secreting Anti-CD42b antibodies are available from Becton Dickinson Immunocytometry Systems, San Jose, CA. Anti-C4d antibodies are available from Quidel Corp. in San Diego, California (#A213) and are generally described in Rogers, J., N. Cooper, *et al.* Complement activation by beta-amyloid in Alzheimer disease. *PNAS* 89:10016-10020, 1992; Schwab, C. *et al.*. Neurofibrillary tangles of Guam Parkinson-dementia are associated with reactive microglia and complement proteins. *Brain Res* 707(2):196 1996; Gemmell, C. A flow cytometric immunoassay to quantify adsorption of complement activation products on artificial surfaces. *J Biomed Mater Res* 37:474-480, 1997; and, Stoltzner, S.E., *et al.* Temporal accrual of complement proteins in amyloid plaques in patients with Down's syndrome with Alzheimer's disease. *Am J Path* 156:489-499, 2000.

[29] The determination of the C4d and CD42b values may alternatively be conducted using a number of standard measurement techniques such as ELISA. Instead of fluorescent labels, there may be used labels of other types, such as radioactive and colorimetric labels. If such other types of assays are to be used, the kits will comprise monoclonal antibodies specific for C4d and CD42b conjugated with appropriate labels such as radioactive iodine, avidin, biotin or enzymes such as peroxidase.

Diagnostic methods

[30] Diagnosis of a patient with SLE is carried out by comparing the determination of C4d with a base value or range of values for the quantities of C4d typically present on the surfaces of platelets in normal individuals. In normal individuals, C4d is not present. When using

flow cytometric measurement with indirect immunofluorescence, the MFC of C4d on platelets of healthy individuals ranged from –1.17 to 0.87 (mean –0.39). (Table I and Table IV). The MFC of platelet C4d in patients having SLE was higher than that of healthy individuals and ranged from –0.85 to 31.67 (mean 2.65). (Table II and Table IV).

Monitoring of patients

[31] A particular feature of the methods of this invention is to indicate or reflect SLE activity that has occurred in the patient during the preceding several weeks or even several months. It is possible, using this procedure, to identify the occurrence of a flare-up of SLE during the previous few weeks or possibly even the previous several months due to persistence of C4d deposited on the surface of platelets.

Automation and computer software

[32] The determinations of C4d and the diagnostic and disease activity monitoring methods described above can be carried out manually, but often are conveniently carried out using an automated system and/or equipment, in which the blood sample is analyzed automatically to make the necessary determination or determinations, and the comparison with the base or reference value is carried out atuomatically, using computer software appropriate to that purpose.

[33] Thus, in one aspect, the invention comprises a method for diagnosing or monitoring systemic lupus erythematosus in an individual comprising (a) automatically determining, in a blood sample from the individual containing platelets, complement component C4d deposited on surfaces of platelets in the sample, and (b) automatically comparing said determinations with reference values for component C4d on surfaces of platelets.

[34] Computer software, or computer-readable media for use in the methods of this invention include:

(1): a computer readable medium, comprising:

(a) code for receiving data corresponding to a determination of complement component C4d deposited on surfaces of platelets;

(b) code for retrieving a reference value for complement component C4d deposited on surfaces of platelets of individuals; and

(c) code for comparing the data in (a) with the reference value of (b).

[35] In embodiments of the invention, one or more reference values may be stored in a memory associated with a digital computer. After data corresponding to a determination of

complement C4d is obtained (e.g., from an appropriate analytical instrument), the digital computer may compare the C4d data with one or more appropriate reference values. After this comparison takes place, the digital computer can automatically determine if the data corresponding to the determination of complement C4d is associated with SLE.

[37] Accordingly, some embodiments of the invention may be embodied by computer code that is executed by a digital computer. The digital computer may be a micro, mini or large frame computer using any standard or specialized operating system such as a Windows™ based operating system. The code may be stored on any suitable computer readable media. Examples of computer readable media include magnetic, electronic, or optical disks, tapes, sticks, chips, etc. The code may also be written by those of ordinary skill in the art and in any suitable computer programming language including, C, C++, etc.

[38] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[39] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[40] The following examples are provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical parameters which could be changed or modified to yield essentially similar results.

EXAMPLES AND EXPERIMENTAL DATA

[41] The following examples are provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

Example 1: Assays of Platelet C4d in Healthy Controls: Negative

[42] Twenty five healthy individuals were studied. As shown in Table I, C4d was not detected on platelets of each of the twenty five healthy individuals. Samples of 1 mL of EDTA-anticoagulated peripheral blood were taken from each individual and used as a source of platelets. The platelets were washed and resuspended in FACS buffer. Levels of C4d and

CD42b were measured by two color indirect immunofluorescence using monoclonal antibodies specific for C4d and CD42b, respectively. Levels of C4d and CD42b are quantitated by flow cytometry using a FACSCalibur cytometer (Becton Dickinson). The platelets were identified by forward and side scatter and CD42b-fluorescence, and the mean fluorescence channel (MFC) was determined for C4d.

[43] More particularly, blood was drawn into 4 cc Vacutainer tubes containing 7.2 mg EDTA as an anticoagulant (Becton Dickinson, Franklin Lakes, NJ), and processed within two hours. Whole blood was diluted 1/10 in phosphate buffered saline (PBS). 10 µl aliquots of the diluted blood were immufluorescently labeled for flow cytometry with 0.25 µg of PE-labeled anti-CD42b monoclonal antibody (Becton Dickinson Immunocytometry Systems, San Jose, CA) to identify platelets, and 0.25 µg of one of the following monoclonal antibodies conjugated to Alexa Fluor 488 (Molecular Probes, Eugene, OR): anti-C4d, anti-C3d (Quidel Corp., San Diego, CA), or the isotype control MOPC21. Samples were incubated 10 min at room temperature, then diluted with 0.5 ml cold PBS and analyzed on a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Platelets were electronically gated by forward scatter properties and expression of CD42b, a platelet-specific marker. Nonspecific binding of immunoglobulins to platelets was determined by performing identical assays in parallel using the isotype control antibody MOPC21 (obtained from ATCC). Specific binding of anti-C4d and anti-CD42b were determined by subtracting the MFC obtained with MOPC21 from the MFC obtained with anti-C4d and anti-CD42b respectively.

Example 2: Assays of Platelet C4d to Distinguish Patients with SLE from Healthy Controls

[44] This example describes conducting assays on patients to diagnose systemic lupus erythematosus, and to establish reference values or ranges of values for complement component C4d.

[45] For this purpose, we recruited 115 patients with lupus from our outpatient office. A single determination of platelet C4d was made in 115 individuals who met ACR criteria for the diagnosis of SLE (Table II) and in 35 healthy controls (Table I). The mean and median values of CR1 and C4d for patients with SLE and healthy controls are shown in Table IV. Whereas the mean value for C4d in healthy individuals was –0.39, the mean value for C4d among patients with SLE was 2.65 ($p = 0.0001$).

Table I. Healthy Controls (n=25)

Mean MFC= - 0.39 Range(–1.17) – (+ 0.87)

Platelet C4d	MFC
2003	-0.28
2005	-0.23
2006	-0.51
2007	-0.05
2008	0.20
2009	0.15
2010	-0.39
2011	-0.71
2013	-0.96
2017	0.87
2020	-0.29
2021	-0.56
2022	0.38
2025	-0.73
2026	-0.24
2027	-0.34
2028	-0.74
2029	-0.05
2030	-0.51
2031	-1.03
2032	-0.42
2034	-0.71
2035	-0.86
2036	-0.48
2037	-1.17

Table II. SLE Patients (n=115)

MEAN = 2.65 RANGE= (-0.85) – (+31.67)

Patient ID	Platelet C4d
1001	3.79
1002	0.59
1003	1.36
1006	4.64
1008	0.00
1009	4.58
1010	20.82
1011	0.74
1012	1.47

1013	10.37
1014	-0.40
1015	9.44
1016	4.18
1017	1.05
1018	1.37
1019	0.00
1021	0.92
1027	0.00
1030	2.36
1031	2.76
1032	0.38
1034	0.00
1035	0.14
1036	7.11
1037	0.00
1038	0.70
1039	12.60
1043	0.00
1044	0.94
1045	0.00
1046	0.40
1047	1.52
1048	0.00
1050	3.80
1052	5.92
1053	1.11
1055	1.24
1056	14.90
1057	0.00
1059	0.03
1060	0.00
1061	4.41
1063	0.84
1064	-0.15
1066	31.67
1067	0.18
1068	2.63
1071	0.03
1072	0.00
1073	1.61
1074	0.02
1075	23.61
1076	8.60
1078	-0.17
1079	1.61
1080	2.59
1081	6.59
1082	2.52
1083	0.05

1084	0.06
1085	4.48
1086	-0.06
1087	0.58
1089	-0.10
1090	9.71
1091	-0.14
1092	-0.38
1093	0.34
1094	-0.53
1095	-0.11
1096	-0.85
1097	-0.12
1098	-0.24
1099	-0.31
1100	0.52
1101	1.49
1102	8.74
1103	-0.35
1104	-0.22
1105	-0.31
1106	0.90
1108	0.89
1109	1.06
1110	2.69
1111	0.59
1112	1.53
1113	1.03
1115	0.92
1116	2.16
1117	1.26
1118	4.97
1119	0.62
1120	0.94
1121	6.17
1122	3.75
1123	1.78
1124	1.20
1125	3.10
1126	2.57
1127	1.47
1128	1.67
1129	1.29
1130	6.35
1131	2.36
1132	7.25
1134	1.47
1135	0.86
1136	1.70
1137	2.70

1138	0.92
1139	1.30
1140	1.34
1141	1.09
1142	3.82
1143	1.60

[46] In SLE patients compared to healthy controls, the sensitivity and specificity of these measures were 39% and 100%, respectively (Table V).

Example 3: Assay of Platelet C4d for Distinguishing Patients with SLE from Patients with Other Diseases

[47] These studies of patients with SLE vs. healthy controls were followed by studies to compare patients with SLE with patients diagnosed with diseases other than SLE (n=103). For this comparison, we studied patients with systemic sclerosis (n=13), rheumatoid arthritis (n=17), osteoarthritis (n=2), hepatitis C virus infection (n=14), polymyositis/dermatomyositis (n=18), Sjogren's syndrome (n=2), urticarial vasculitis (n=1), sickle cell anemia (n=8), overlap syndrome/undifferentiated connective tissue disease (n=12), leukemia/lymphoma (n=9), primary Raynaud's syndrome (n=3), hemophilia (n=2), and psoriatic arthritis (n=2). A single determination of platelet C4d was made, using the same assay. The mean and median values of C4d for patients with SLE, as compared with patients with other diseases are shown in Table IV. Whereas the mean value for C4d in patients with other diseases was 0.53, the mean value for C4d among patients with SLE was 2.59 (p= 0.0001). In SLE patients compared to patients with other diseases, the sensitivity and specificity of these measures were 39% and 94%, respectively (Table V).

Table III. Other Diseases (n=103)

MEAN = 0.68 RANGE = (-3.29) - (+10.22)

Patient ID	**Platelet C4d**
3003	1.11
3004	0.42
3014	0.66
3021	0.17
3022	0.28
3023	0.00
3028	0.56

3029	0.53
3030	1.03
3031	10.22
3034	1.02
3035	1.15
3036	1.33
4001	0.08
4002	0.73
4008	0.91
4011	1.07
4018	0.02
4019	1.04
4020	0.20
4021	0.44
4022	0.10
4023	1.17
4024	0.03
4025	0.28
4026	0.32
4027	0.06
4028	1.22
4030	0.53
4033	1.03
4034	1.15
5001	1.05
5003	0.55
6001	2.12
6002	1.04
6003	0.47
6004	0.50
6005	0.04
6006	0.40
6007	0.04
6008	0.18
6009	0.11
6010	3.58
6011	0.14
6012	0.40
6013	0.71
6014	0.21
6015	1.28
6016	1.01
6017	2.00
8001	0.54
8002	1.46
8003	0.05
8004	1.39
8005	1.83
8007	0.03
8008	0.02

[redacted]

8009	0.10
8012	0.29
8013	0.34
8015	0.56
8018	0.24
8019	0.05
8020	0.25
10001	0.89
12001	0.29
12002	0.06
12003	0.16
12004	0.46
12005	0.33
12006	0.05
12007	0.14
12008	0.10
13003	1.37
13007	0.46
13008	0.08
13009	0.47
13010	0.13
13011	1.45
13012	0.15
13013	0.42
13014	0.20
13015	1.04
13016	0.97
13017	0.58
14001	1.15
14002	1.63
14003	0.52
14004	0.67
14005	1.25
14006	1.47
14007	0.17
14008	0.14
14009	0.05
15002	0.11
15003	0.40
15004	0.89
16001	1.06
16002	0.14
17001	-3.29
17002	0.17
18001	0.80
18002	1.00

Example 4: Assays of CR1 and C4d for Measuring Disease Activity in Patients with SLE

[48] We then examined the utility of platelet C4d levels in measuring disease activity as defined by the Systemic Lupus Erythematosus Disease Activity Indes (SLEDAI). We present the results of the first 115 lupus patients entered into the study. Using a univariate linear regression model, platelet C4d was the most significant predictor of disease activity (P=0.002).

Table IV. Analysis of Platelet C4d

Platelet-C4d

		Mean	Standard Deviation	Median
SLE	(n=115)	2.65	4.76	2.13
Healthy Controls	(n=25)	-0.39	0.46	-0.42
Other Diseases	(n=103)	0.68	1.18	0.46

Comparison	C4 (p values)
SLE vs Healthy Controls	0.0001
SLE vs Other Diseases	0.0001

Table V. Analysis of Platelet C4d

Comparison	Sensitivity	Specificity
SLE vs Healthy Control	.39	1.00
SLE vs Other Diseases	.39	.94

[49] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the techniques of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for diagnosing systemic lupus erythematosus in an individual, comprising (a) determining, in a blood sample from the individual containing platelets, complement component C4d on surfaces of platelets in the sample, and (b) comparing said determinations with, respectively, the quantities of component C4d deposited on surfaces of platelets of individuals not having systemic lupus erythematosus.

2. A method according to claim 1 in which the determinations of C4d and CD42b are conducted by a method comprising binding the C4d to a conjugate of a monoclonal antibody specific for component C4d with a first labeled moiety, binding the CD42b to a conjugate of a monoclonal antibody specific for CD42b with a second labeled moiety, and determining the first and second labeled moieties.

3. A method according to Claim 2 in which the labeled moieties are fluorescent moieties.

4. A method according to claim 3 in which the fluorescent moietics are determined by determining the mean fluorescence channel using flow cytometric analysis.

5. A method of monitoring disease activity of systemic lupus erythematosus in an individual, comprising (a) determining, in a blood sample from the individual containing platelets, complement component C4d deposited on surfaces of the platelets in the sample, and (b) comparing said determinations with, respectively, the quantities of component C4d deposited on surfaces of platelets previously obtained from the individual.

6. A method of diagnosing systemic lupus erythematosus in an individual, comprising (a) determining, in a blood sample from the individual containing platelets, complement component C4d deposited on surfaces of platelets in the sample, and (b) comparing said determination with the quantity of component C4d deposited on surfaces of platelets of individuals not having systemic lupus erythematosus.

7. A method according to claim 6 in which the determination of C4d is conducted by a method comprising binding the C4d to a conjugate of a monoclonal antibody specific for C4d with a labeled moiety, and determining the labeled moiety.

8. A method according to Claim 7 in which the labeled moiety is a fluorescent moiety.

9. A method according to claim 8 in which the fluorescent moiety is determined by determining the mean fluorescence channel using flow cytometric analysis.

10. A method of monitoring disease activity of systemic lupus erythematosus in an individual, comprising (a) determining, in a blood sample from the individual containing platelets, complement component C4d deposited on surfaces of the platelets in the sample, and (b) comparing said determination with the quantity of component C4d deposited on surfaces of platelets previously obtained from the individual.

11. A method according to claim 10 in which the determination of C4d is conducted by a method comprising binding the C4d to a conjugate of a monoclonal antibody specific for C4d with a labeled moiety, and determining the first labeled moiety.

12. A method according to Claim 11 in which the labeled moiety is a fluorescent moiety.

13. A method according to claim 12 in which the fluorescent moiety is determined by determining the mean fluorescence channel using flow cytometric analysis.

14. A method for diagnosing or monitoring systemic lupus erythematosus in an individual comprising (a) automatically determining, in a blood sample from the individual containing platelets, complement component C4d deposited on surfaces of the platelets in the sample, and (b) automatically comparing said determination with a reference value for component C4d deposited on surfaces of platelets.

15. A kit for diagnosing or monitoring systemic lupus erythematosus in an individual, comprising a conjugate of a monoclonal antibody specific for complement component C4d with a fluorescent moiety

16. A computer readable medium, comprising:

(a) code for receiving data corresponding to a determination of complement component C4d deposited on surfaces of platelets;

(b) code for retrieving a reference value for complement component C4d deposited on surfaces of platelets of individuals;

(c) code for comparing the data in (a) with the reference value in (b).

DIAGNOSIS AND MONITORING OF SYSTEMIC LUPUS ERYTHEMATOSUS

ABSTRACT OF THE DISCLOSURE

Methods for diagnosing and monitoring systemic lupus erythematosus (SLE) by determining, in a blood sample from the individual being diagnosed or monitored, complement component C4d deposited on surfaces of platelets in the sample. For diagnosis this is compared with the quantity of C4d present on platelets of normal individuals. For monitoring it is compared with a value in a sample or samples previously obtained from the individual patient.